INTERACTION OF GULF COAST TICK, *Amblyomma maculatum* KOCH, NYMPHS ON CATTLE

A Thesis

by

AARON WEXLER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Entomology

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Approved by:

Chair of Committee,	Pete D. Teel
Committee Members,	Karen F. Snowden
	Jimmy K. Olson
	Michael T. Longnecker
Head of Department,	Kevin M. Heinz

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ABSTRACT

Interaction of Gulf Coast Tick, *Amblyomma maculatum* Koch,

Nymphs on Cattle. (August 2005)

Aaron Wexler, B.S., George Washington University

Chair of Advisory Committee: Dr. Pete D. Teel

Concern over the vector potential of the Gulf Coast tick, *Amblyomma maculatum* Koch, with the pathogen *Ehrlichia ruminantium* Dumler, causative agent of the disease heartwater, has increased the need for fundamental knowledge of tick ecology and behavior, specifically immature tick biology. Texas strain *A. maculatum* adult male ticks, known to emit attraction-aggregation-attachment pheromone (AAAP), were used to artificially simulate immature tick interaction with adults, in forced environments, on cattle. Artificial areas were grouped by treatment level, which were 1) aggregating, attached adult males, 2) aggregating attached adult females or 3) an empty area with no adults, as a control. Immature ticks were noted to be 6 times more likely to be aggregating in the AAAP treatment area when adult males were present. In the presences of either adult female ticks or no ticks at all, immature ticks were found to be attaching at random within the given area were they where permitted to feed. A second correlation of mortality was noted among immature ticks in the presence of AAAP emitting adult male ticks. In the permitted area where immature ticks could attach and feed, immature ticks were twice as likely to have survived to engorgement if adult male ticks were present in the area as well (53%). There was no difference in the survival rate among immature ticks if adult females were present or no adults at all, 26% and 21%, respectively. The

study demonstrated that a significant attraction existed between immature ticks and attached adult males emitting AAAP.

DEDICATION

Wealth is defined in many ways. Some define it by how much money you have, some by how many children, some by who you are in the community; the list goes on. For me, wealth is defined by family and friends and the joy that they have brought to my life. Although I do not have much blood family in Texas, I have had the blessed experience to develop friendships with a number of unique individuals while I have spent my time here. I feel that I have found a family. Graduate school and academia in general seems to engender familial bonds that fill the void of family who are often at a great distance for many of us. I would like to thank all of you for appreciating who I am and allowing me to grow in your company. Although we don't get paid very much, I feel that each semester has increased my wealth ten-fold. I leave Texas a rich man, something that I never thought I would ever do while in grad school, and hope that any experience I have in the future will parallel such grandeur.

This paper is dedicated to all those that have made me feel like a king, if you are a friend, you know who you are.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Teel. Dr. Teel is both inspirational and modestly respectful of individuality. He knows what questions to ask, what answers to give and how much space a graduate student requires in order to develop personal skills and style. Dr. Teel has been a wonderful academic advisor.

Secondly, I would like thank Otto Strey. Otto is the core of the Texas A&M Tick Research Laboratory. Without Otto, tick research at A&M would not survive in a progressive manner. Not only this, but Otto has more than assisted the success of my research. I owe a debt of gratitude to Otto, a gentleman and dedicated researcher.

I would like to thank my committee. Each member has encouraged my work and did so in a personal way. I thank them for the time and assistance.

I would also like to thank my lab mate Hee for being a great co-worker who was always at my service and often with a different opinion that I could appreciate. I must thank Andrew and Jeremy for their care of the tick lab and their assistance. I would like to thank Dr. Cognato for his wise advice and Dr. Keeley for the opportunity to attend his class and continue as his laboratory instructor.

Almost finally, I would like thank my wife Ngozi, for her encouragement and ability to make me smile, even after I drove three hours each way (50,000+ miles to and from College Station and San Antonio), while she was finishing a 100+ hour work week.

Lastly, I must thank all the ticks, cows and chickens that served as laboratory subjects. I realize that they can't hear me, but I believe it is important to acknowledge those who are forced into the service of humans. We owe them all a debt of gratitude.

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CHAPTER I

INTRODUCTION

Gulf Coast ticks are significant pests of pastured cattle that require new strategies of population surveillance and control. *Amblyomma maculatum* Koch not only presents considerable economic losses to the United States cattle industry, but also is a recognized vector of heartwater, *Ehrlichia ruminantium* Dumler (formerly *Cowdria ruminantium* Moshkovski, *Rickettsia ruminantium* Cowdry), a lethal disease of emerging importance. In the case of heartwater, both disease and vector are currently increasing their geographic range. Evidence suggests that *A. maculatum* is spreading inland, while heartwater is spreading globally among naïve bovine populations.

Male Gulf Coast ticks are known to emit attraction-aggregation-attachment pheromone (AAAP) that elicits attraction and attachment responses among males and females (Gladney et al. 1974a and 1974b). The pheromone is also thought to attract and initiate an attraction-aggregation-attachment response from nymphs (Williams 2002) as Bryson et al. (2000) found with *A. hebraeum* Koch. This study tested the hypothesis that AAAP or other stimuli from the adult male ticks attract the immature stages of the Gulf Coast tick as well as provide fundamental ecological information of the on-host phase of the adults and immature stages. Data from this study initiated the first attempt to provide primary knowledge in developing a reliable surveillance technique to find immature stages in the field. Such information is crucial in developing new tactics for Gulf Coast tick surveillance and suppression, and in developing new strategies to prevent young ticks from maturing into reproductive adults.

This thesis follows the style and format of the Journal of Medical Entomology.

Objectives

1. Illustrate on-cattle characteristic activity of juvenile stages of Gulf Coast ticks.
2. Determine the characteristic attraction-aggregation-attachment behavior of immature stages in the presence of attached adults.

The Problem, Background, and Justification

Gulf Coast ticks (Figure 1) are traditionally considered parasites of pastured cattle along the Gulf and Atlantic coasts from Georgia to Texas roughly 160-240 km inland from the coast (Bishop and Hixson 1936; Cooley and Kohls 1944; Bishop and Trembley 1945). However, the current range of Gulf Coast ticks extends further north along the Atlantic coast southern New Jersey and now includes an expanding inland population west central Texas including areas of Oklahoma and Kansas (Figure 2). The tick is also found throughout Central America, Jamaica and Columbia (Williams 2002 citing Bishop and Trembley 1945; Walker and Olwage 1987). Among cattle, infestation by Gulf Coast ticks causes weight loss, anemia and stress. Feeding lesions also create secondary infection sites leading to bacterial infection and infestation by primary and secondary screw worms (*Cochliomyia hominivorax* Coquerel and *C. macellaria* Fabricius respectively) (Harwood and James 1979). Economic data suggest that Gulf Coast ticks currently cost the cattle industry over $3.7 million a year. This figure is estimated from Drummond (1987) and does not include current herd sizes or the expanded range of the tick, which would greatly inflate the actual cost to the industry.

Generally, control of Gulf Coast ticks targets the adults that are attacking or have already attached to the host. Adult ticks prefer to aggregate around the ears of cattle. In large numbers, significant inflammation results in an inward curling of the distal end of

the ear due to cartilage deformity (Figure 3: Teel personal communication). As such, adult ticks are much easier to target and control due to their preferred feeding location on cattle. Immature ticks do not share this preference (Figure 3). Immature ticks display a negative geotaxis and seem to prefer the withers, midline and tail-head (Williams 2002). A lack of information on immature ticks, specifically where and how to target them, limits their control and allows unfettered development to adult stages. A greater understanding of the immature stages is required, which may assist in developing strategies that target multiple stages of the tick life cycle.

Gulf Coast ticks have been identified as capable vectors of heartwater, a fatal disease of ungulate populations in Africa and the Caribbean. Heartwater originates from Africa and is a rickettsial infection of livestock, wild birds, mammals, and reptiles; caused by *Ehrlichia ruminantium* (Sonenshine 1991). There is no approved vaccine or practical treatment available for the disease, and the danger that immunologically naïve bovine populations face is a mortality of 60-90% (Williams 2002). Uilenberg (1982) and Jongejan (1991) have established *A. maculatum* as a carrier of *E. ruminantium* in the laboratory setting. More recently, Mahan et al. (2000) have elevated the vector potential status of Gulf Coast ticks using non-clinical animals, a major blow in avoiding establishment of the disease in North America. Burridge et al. (2000) working with Mahan, has also detected evidence of heartwater in *A. sparsum* Neumann, ticks found on tortoises imported into Florida. Where heartwater is known to occur in the New World, *A. variegatum* Fabricius is the only vector that exists in the same geographic range (in the Caribbean). Of the twelve known vectors, *A. maculatum* is the most widely distributed in

Figure 1: Gulf Coast tick, *Amblyomma maculatum* Koch, adults, male (right) and female (left). Note the distinctive sclerotization on the entire dorsal body surface on the male compared with the reduced area of the female.

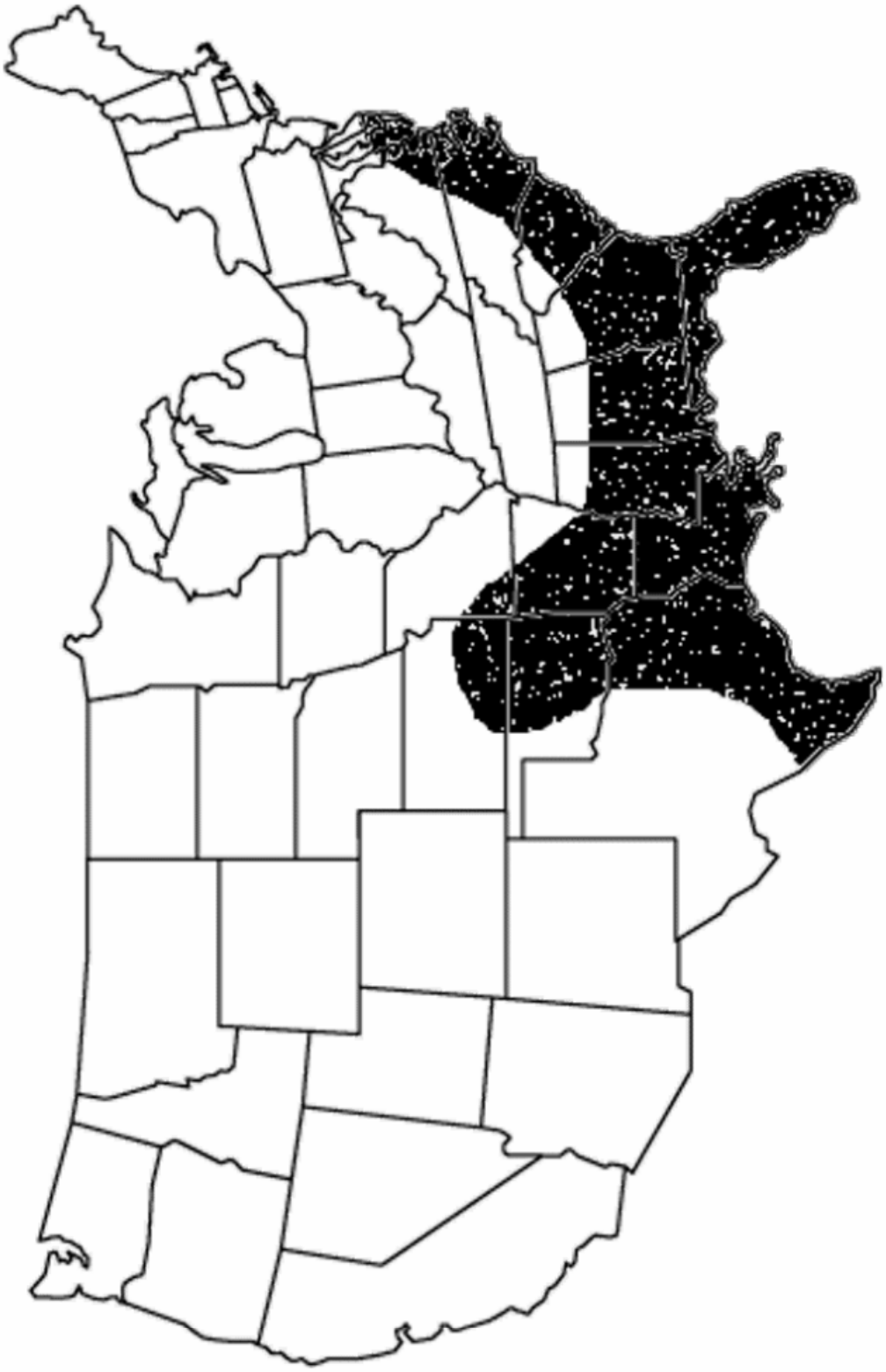

Figure 2: The current range of the Gulf Coast tick, *Amblyomma maculatum* Koch, in the United States.

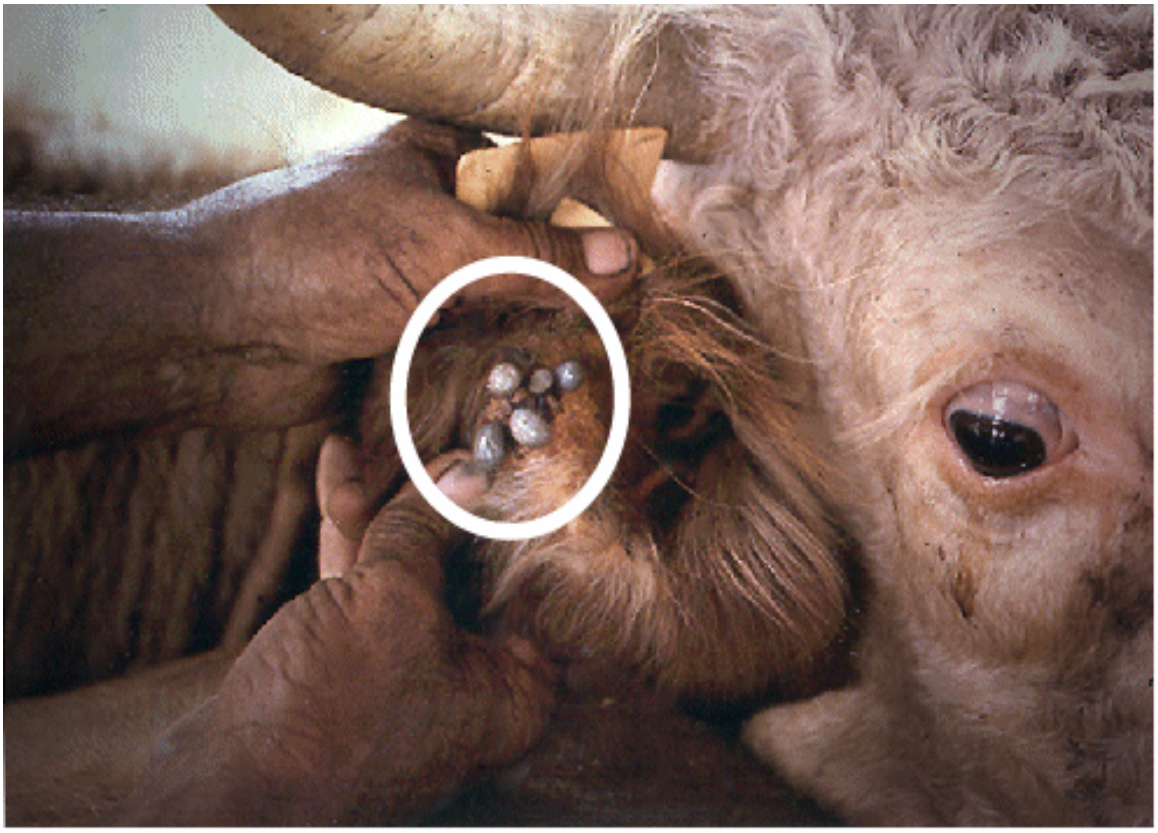

Figure 3: Aggregation of engorged Gulf Coast ticks, *Amblyomma maculatum* Koch, in typical location on cattle, on or around ears (Photograph by P. D. Teel 1981). As females feed, their body size increases dramatically while males remain relatively unchanged, making males difficult to see from casual observation.

the United States and the geographic range of this tick is increasing annually (Williams 2002). As eradication of the Gulf Coast tick seems unlikely, suppression and monitoring of populations is necessary to prevent or contain any outbreaks among domestic or wild populations in the United States, increasing the need for new surveillance techniques for Gulf Coast ticks.

The Gulf Coast tick is a 3-host parasite that must find and feed on fairly specific hosts for each of three post-embryonic stages (Figure 4); adults and immature ticks have a moderately limited host selection (Hoogstraal and Aeschliman 1982). Engorged adults are found on a variety of vertebrates, including cattle, sheep, swine, white-tailed deer, coyotes, raccoons and birds (Sonenshine 1991; Barker et al. 2004). It is known that larvae and nymphs commonly feed on ground dwelling birds, small mammals and less commonly reptiles (Barker et al. 2004, Teel et al. unpublished). Nymphs are known anecdotally to parasitize larger vertebrates (anecdotal note in Uilenberg 1982), but reviews are scant and the degree to how often these hosts are exploited is unknown. Immature stages are relatively small and are commonly indistinguishable between species, often leading to species misidentification of *A. maculatum*. Juveniles have been observed to feed on cattle in the laboratory, but in depth analysis is severely limited. Within the literature, the only study of immature host interaction is Williams' investigation of preferred attachment sites on cattle (Williams et al. in press). In the same study, Williams found an aggregation of nymphs around an engorged male mistakenly introduced on a cow (Williams et al. in press). This seemingly incidental event hinted that immature ticks may be attracted to adult male pheromone.

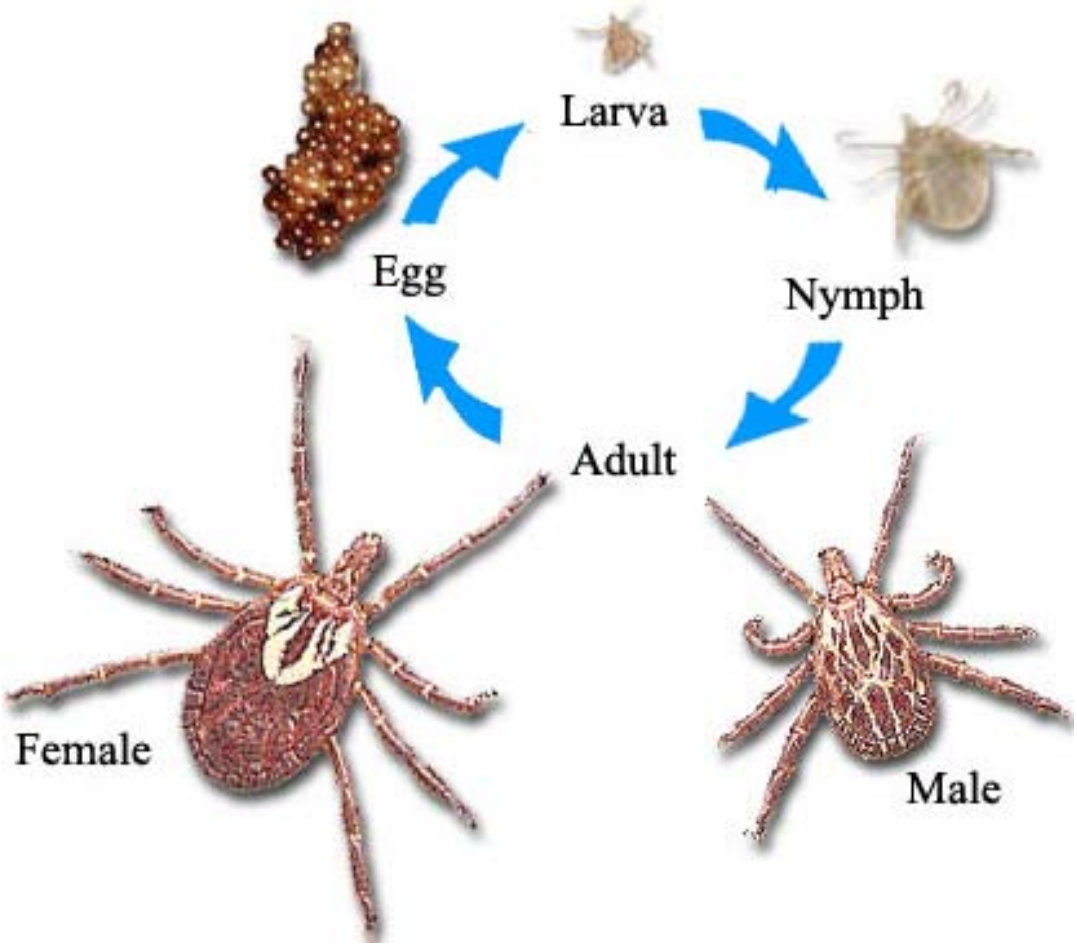

Figure 4: Three-host tick life cycle of Gulf Coast tick, *Amblyomma maculatum* Koch, egg and three post-embryonic stages.

Commonly among ticks, the adult females attract males by emitting a sex pheromone, as do lone star ticks, *Amblyomma americanum* (Kellum and Berger 1977). However, among a few *Amblyomma* spp. (*A. hebraeum*, *A. maculatum*), the male emits the pheromone attracting the female. Feeding *A. hebraeum* male ticks are known to attract unfed, newly-attached adult females and nymphs in the environment from a combination of AAAP and carbon dioxide exhaled by their hosts (Bryson et al. 2000). It is therefore recognized that immature stages are attracted to AAAP emitted from engorged adult males in a closely related species of the Gulf Coast tick. However, without experimental verification, this does not necessarily mean this behavior occurs in Gulf Coast ticks.

The Gulf Coast tick is univoltine and each developmental stage has a typical period for seasonal activity that may or may not overlap with the two immature stages, depending on geographic location of tick, i.e. evidence suggests that there are genetically distinct populations (Figure 5: Williams 2002). Oklahoma and Kansas adult Gulf Coast tick populations are active from February to August, with a peak in late March, and continuing into April (Teel et al. 1998, Williams & Hair 1976). Larvae and nymphs are increasingly observed on bird populations from June to August (Semtner and Hair 1973, Williams and Hair 1976). Within the coastal states including Texas, Gulf Coast tick adult seasonal activity occurs in the late summer and early fall months, with a peak in mid-September (Hixson 1940, Teel et al. 1998, Williams 2002). The larvae and nymphs are most abundant in January and February, respectively (Teel et al. 1998, Williams 2002). There is little seasonal overlap between adults and juveniles of Texas populations, but

there is quite an overlap between all stages of the Oklahoma and Kansas population. This seasonal overlap can provide a field example of how adults and AAAP may be influencing immature populations.

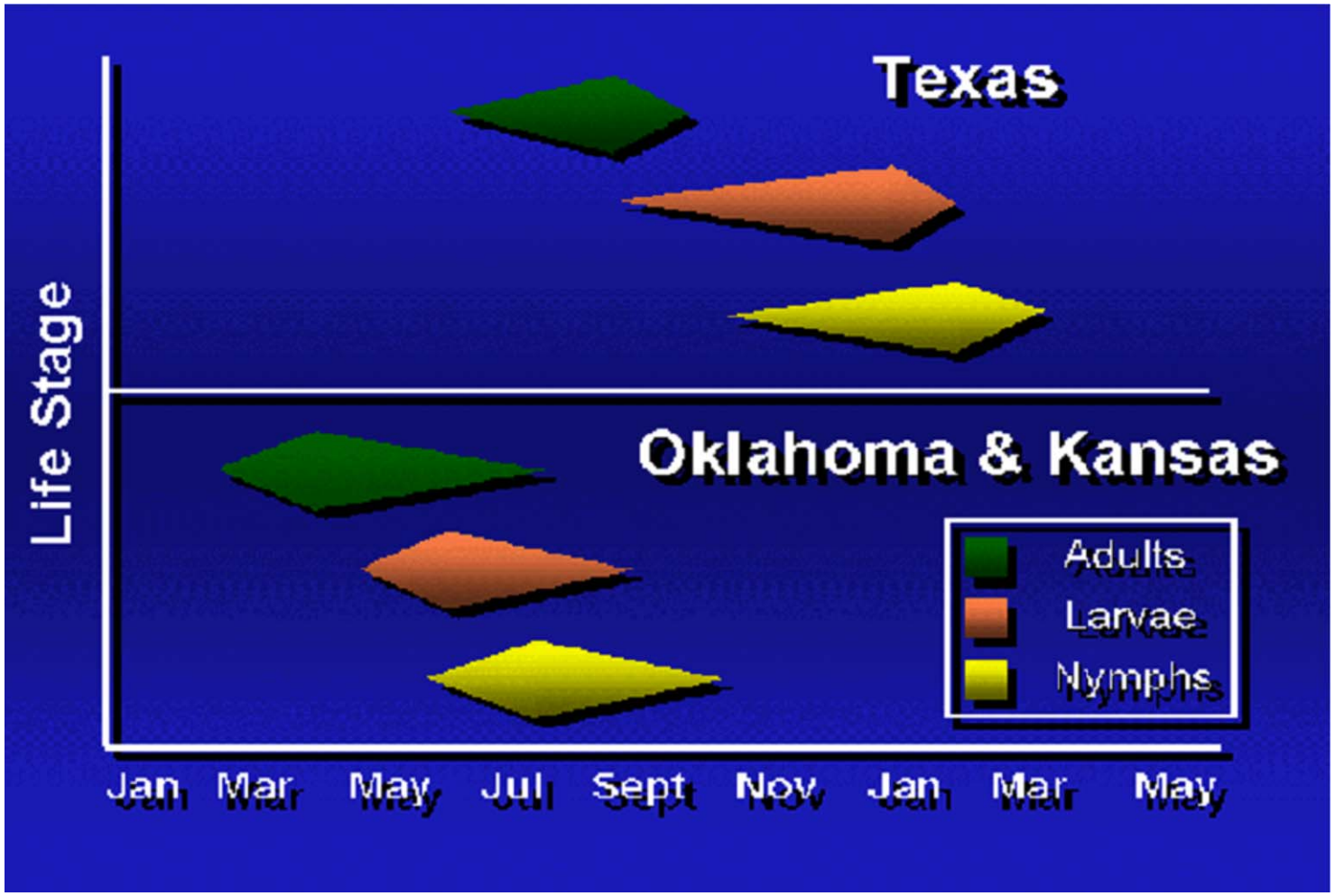

Figure 5: Seasonal phenologies of adult, larval, and nymphal Gulf Coast tick, *Amblyomma maculatum* Koch, populations in Texas and Oklahoma – Kansas (Williams 2002).

CHAPTER II

PRELIMINARY INVESTIGATION OF ATTRACTION, AGGREGATION AND ATTACHMENT PREFERENCES OF GULF COAST TICK, *Amblyomma maculatum* KOCH, NYMPHS, TEXAS AND OKLAHOMA STRAINS ON CATTLE

Investigating whether or not immature Gulf Coast ticks aggregate on a host and in what numbers would further studies in immature tick surveillance and control. Although it is published that immature ticks have a greater propensity to attack small hosts, it is this study's working hypothesis that immature ticks do attack large hosts in the field, but difficulties obtaining field data have prevented its proof thus far. Nymphs and adults share a number of mammalian hosts, and although it seems incidental, they also attack humans (Felz et al 1996). In the field, male Gulf Coast ticks aggregate and attach before females. Feeding males emit AAAP which attract females and other males to aggregate and attach in close proximity (Gladney et al 1974a and 1974b). A feeding period of 4 to 6 days is required for male ticks to begin production and release of AAAP (Kim 2004). The purpose of this preliminary study was to develop the technique of using cells of stockinette material on cattle and to determine the necessary sample size of nymphs to infest cells on cattle used to artificially simulate interactions among nymphs and fed males emitting AAAP.

Materials and Methods

Texas and Oklahoma strain Gulf Coast ticks were used in this study. All ticks were fed on Leghorn chickens in the Tick Research Laboratory Veterinary Park of Texas A&M University, College Station, TX 77843, under approved Animal Use Protocol 2002-208, incubated at 23ºC, 85% RH, with a 14-hour photophase. The Texas strain Gulf Coast ticks were collected from Refugio County, TX and the Oklahoma strain were collected from Payne County, OK. Three cows were used in this experiment. The cows were housed in the Tick Research Laboratory. Cows were maintained in metabolism-like elevated stalls and provided with Calf Creep Pellet (Producers Cooperative Association, Bryan, TX 77803) twice daily and given free access to water (Figure 6). Temperature and humidity were maintained at 20-30ºC and 85-95% RH with a 12-hour photophase and monitored hourly with a Hobo® Pro Series (Model No. H08-032-08, Onset Computer Corporation, Pocasset, MA 02559-3450).

Cells on cows infested with Texas and Oklahoma strain Gulf Coast ticks were used to artificially simulate interactions among nymphs and fed males emitting AAAP. Eight cells were arranged on the back of each cow. The first cell was labeled "A", cells and labeling proceeded posteriorly to "H" (Figure 7). Where the cells were to be glued onto the hide, hair was clipped down to ¼ cm. The cell material used was 4 inch wide Tomac® tubular stockinette (American Hospital Supply, McGraw Park, Il 60085) glued to the test animal with livestock identification tag cement (Nasco, Fort Atkinson, WI, 53538). Size 64 rubber bands (Alliance Rubber Company, Hot Springs, AR 71903) were used to close cells. The stockinette material was stretched to create a cell roughly 6 in. in diameter (15.24 cm).

A small cell, roughly 2 in. (5.08 cm) in diameter, of 2 inch wide Tomac® tubular stockinette (American Hospital Supply, McGraw Park, Il 60085), was glued to the test animal with livestock identification tag cement in the center of each outer 6 in. cell (Figure 7). The smaller cells were closed with rubber bands.

Figure 6: Cows housed in the Tick Research Laboratory, maintained in metabolism-like elevated stalls, provided with Calf Creep Pellet twice daily and given free access to water.

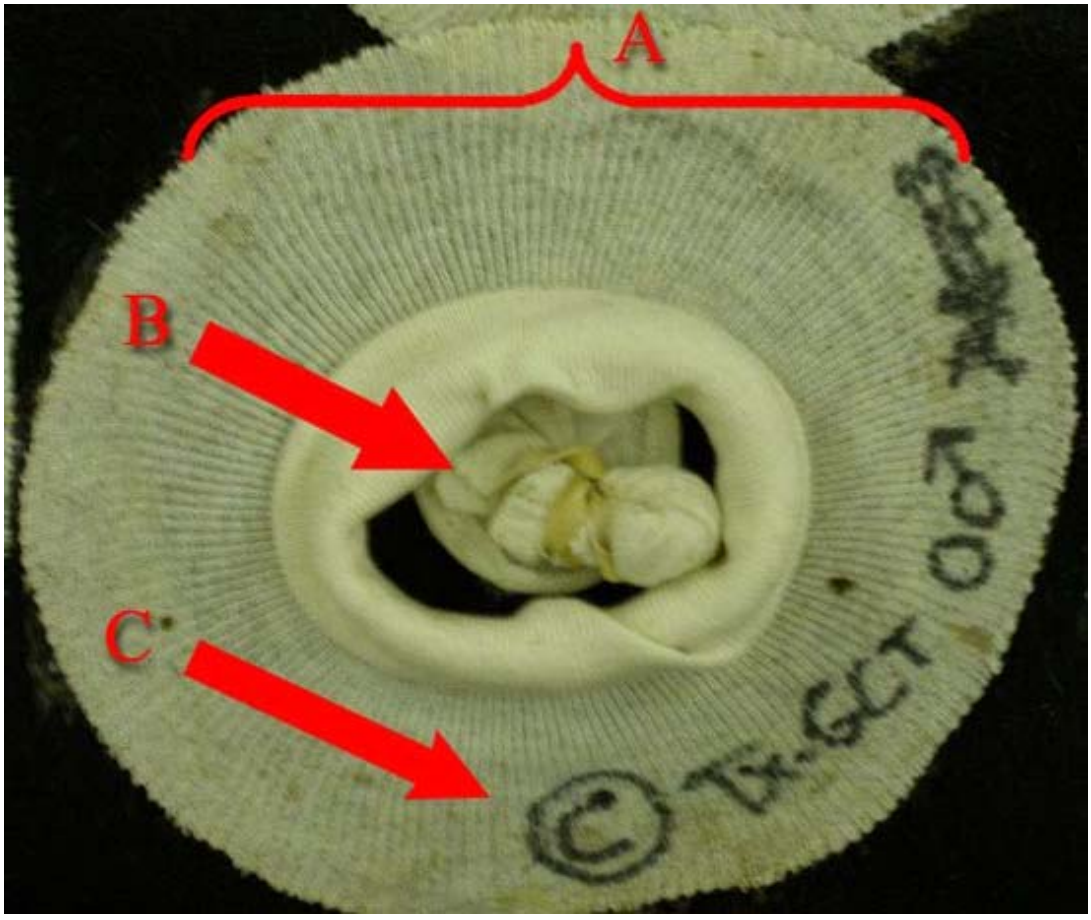

Figure 7: Example of a tick holding cell used in study. A) A 6 in. cell of stockinette material is stretched and glued on the back of a cow. B) A 2 in. cell, of similar stockinet material, used to isolate adult treatments and concentrate presumed AAAP in a specified area, was glued in the center. C) Cells were labeled alphabetically, by strain, and treatment type.

Eight cells were arranged, divided by the midline, on the back of each cow. Texas strain Gulf Coast ticks were on the right side and Oklahoma strain Gulf Coast ticks on the left side. On each cow, ten adult males were used for each of the three treatment cells and none in the appropriate control (Figure 8). Each group of adult ticks was placed in each 2-in. cell, and forced to attach and feed. The smaller cells were used to isolate male ticks within the center of each cell. The inner cell was removed simultaneously with the infestation of immature ticks. The inner cell stockinette was removed by gently pulling it from the hair, creating a ring of bare, exposed skin (Figure 9). Until this time, the adults were forced to attach and feed in the center of the cell. The predetermined period of adult attachment of 5 days was selected based on preliminary results indicating that adult males reach optimal pheromone output after 4-8 days. Thus, when the inner cell was removed and nymphs were released within the cell, the pheromone attractant emanated from the center of the cell. Each control cell also contained the smaller cell in an attempt to mimic any conditions such cells would create during the removal process of the smaller cells.

Nymphs were released into the cell on the 6th day post-release of the adults. The anterior two cells were infested with 30 nymphs each. Proceeding posteriorly, the next two cells (control cells) were infested with 15 nymphs each. The third row of cells were infested with 6 nymphs each. The posterior two cells were infested with 15 nymphs each. Nymphs were released into the cell by placing them into an eppendorf tube, clustering the nymphs by shaking the tube and then tapping them over the center surface of the cell. While this could have produced a bias on initial nymph contact with the skin, it was necessary to facilitate a rapid introduction and reasonable cell closure to prevent

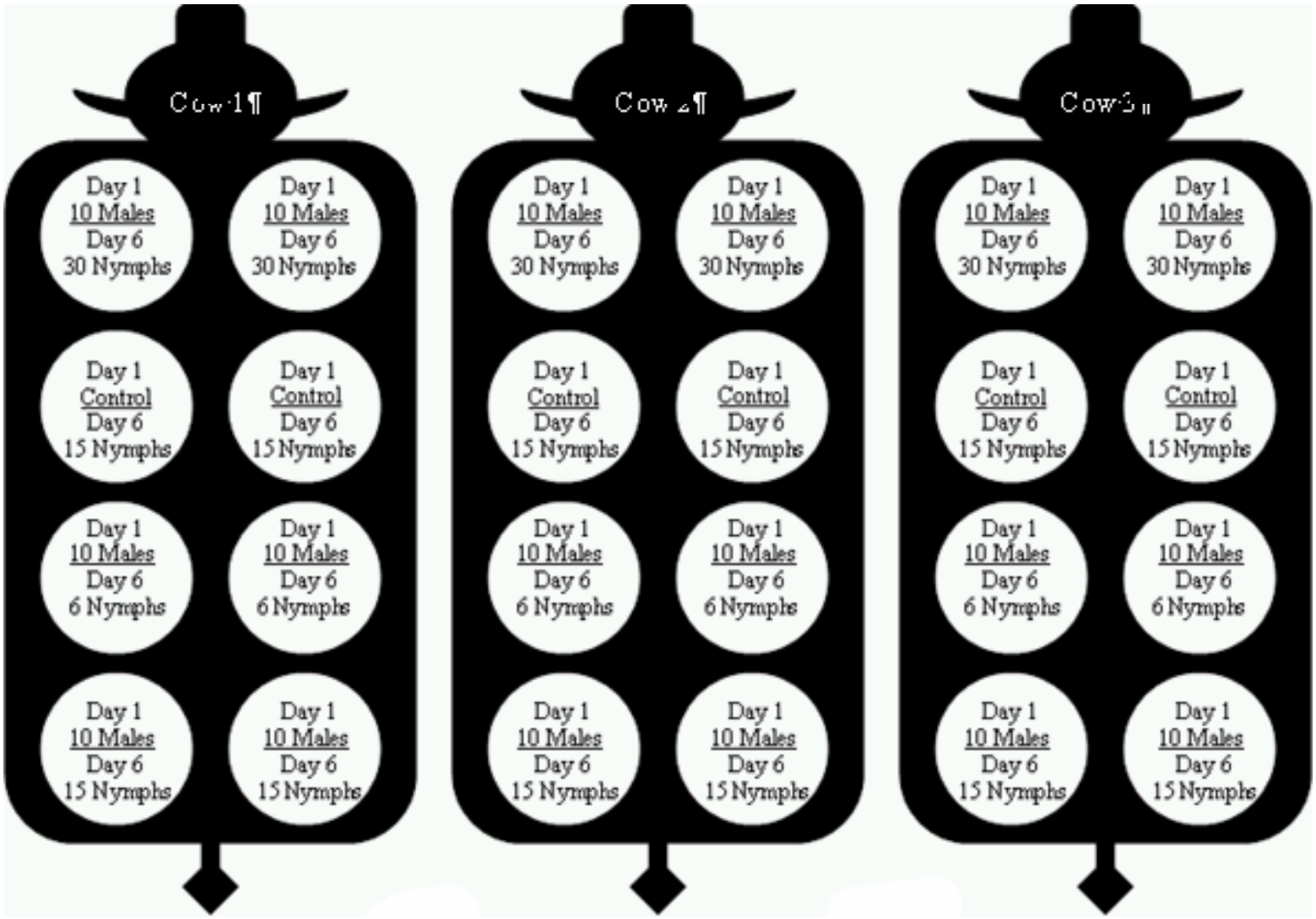

Figure 8: Experimental design of preliminary experiment utilizing three cows with cells arranged on dorsal surface of cows.

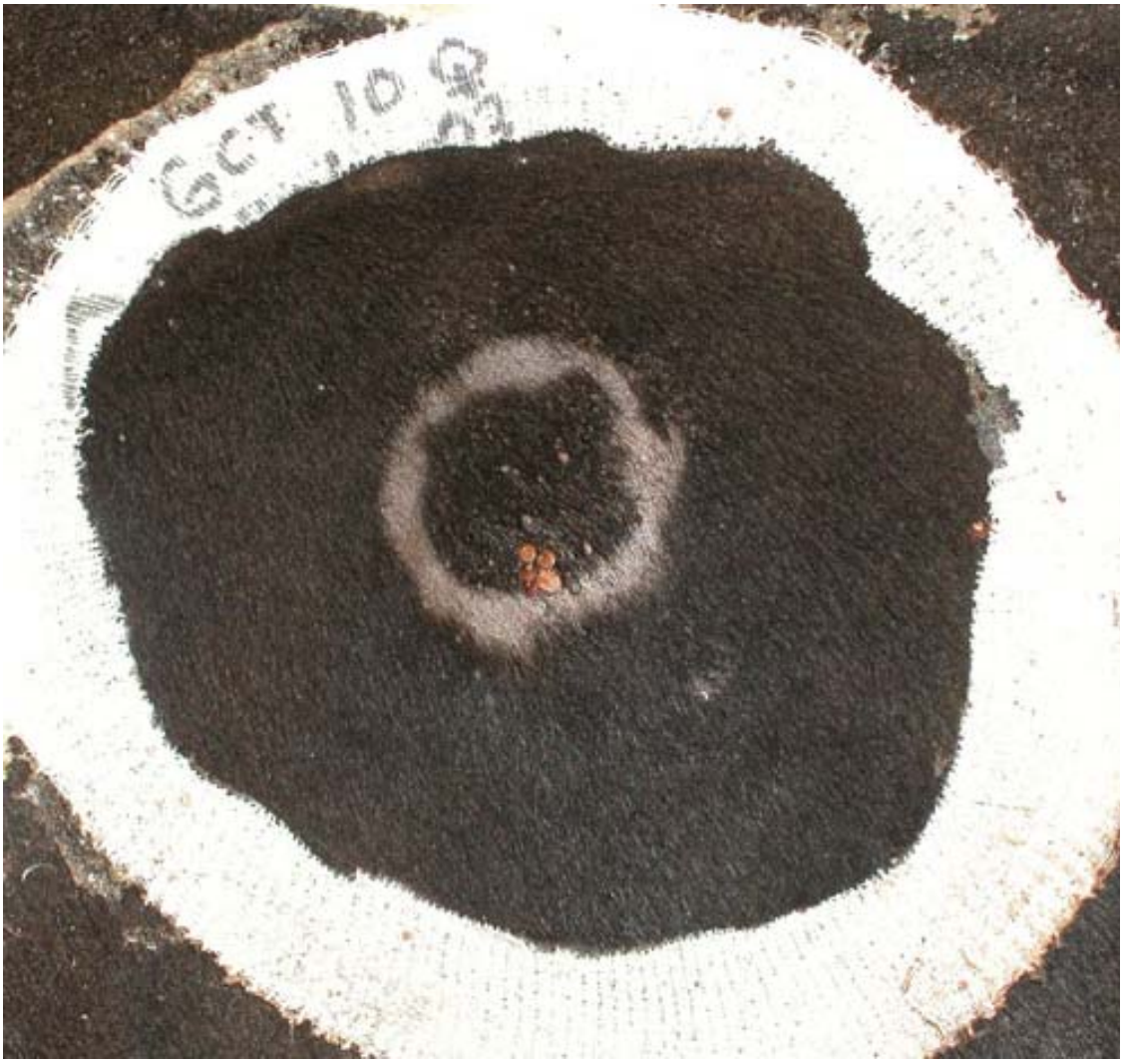

Figure 9: Photograph illustrating ring of bare skin created when inner cell isolating adult Gulf Coast ticks, *Amblyomma maculatum* Koch, is removed from coat of cow.

tick escape. General observations regarding any cell damage or maintenance were made daily before noon (after the removal of the inner cells, day 6).

The experiment was completed on day 12, the 7th day after the release of the nymphs. On the morning of the 7th day after nymphs had been released into each cell, the stockinet material of the outer cell was cut with scissors and observations were made. Nymphs and adults were counted as attached "inside the ring," "on the ring" and "outside the ring."

Nymphs and adults were counted by two methods, visually and manually. A Nikon Coolpix5000 (Nikon Corporation, Tokyo, JAPAN) camera was used to photograph the cell (on the last day only) and the locations of each nymph or adult were then marked onto a printout of that photograph corresponding to what was observed. After the visual count with the camera was completed, the cell was then manually cleared and both immature and adult ticks were counted.

Results

There was no formal analysis the data collected from this preliminary study, and only observational inferences were made. A total of 180 adult male Gulf Coast ticks were used in this experiment, 90 Texas strain and 90 Oklahoma strain (Table 1). After the 12 day study period, 129 (72%) adult males were recovered including 75 (83%) Texas strain and 54 (60%) Oklahoma strain (Table 2). Nymphs (396 total) were free-released into the cells on the 6th day of the study period, 198 Texas strain and 198 Oklahoma strain. Upon recovery of nymphs (168 total, 42% of original), it was noted that the number of Texas strain nymphs recovered was higher than that of Oklahoma, 121 (61%) and 47 (24%) respectively. In every control case, the number of nymphs

Table 1: Data collected from preliminary investigation of aggregation attachment and attachment preferences of Gulf Coast tick, *Amblyomma maculatum* Koch, nymphs, Texas and Oklahoma strains on cattle.

	Cell Name	Males Released	Males Recovered	Nymphs Released	Nymphs Inside	Nymphs Outside	Nymphs Engorged and Dropped	Total Nymphs Recovered
Cow 1	A (TX)	10	10	30	14	1	7	22
	B (OK)	10	6	30	2	1	0	3
	C (TX)	Control	0	15	0	3	0	3
	D (OK)	Control	0	15	0	1	1	2
	E (TX)	10	8	6	1	1	0	2
	F (OK)	10	7	6	0	0	0	0
	G (TX)	10	10	15	10	0	0	10
	H (OK)	10	8	15	0	0	0	0
					27	7	8	42

	Cell Name	Males Released	Males Recovered	Nymphs Released	Nymphs Inside	Nymphs Outside	Nymphs Engorged and Dropped	Total Nymphs Recovered
Cow 2	A (TX)	10	8	30	18	3	6	27
	B (OK)	10	8	30	5	2	12	19
	C (TX)	Control	0	15	1	5	2	8
	D (OK)	Control	0	15	0	1	0	1
	E (TX)	10	10	15	8	0	1	9
	F (OK)	10	5	6	3	1	2	6
	G (TX)	10	8	6	3	0	5	8
	H (OK)	10	3	15	2	1	0	3
					40	13	28	81

	Cell Name	Males Released	Males Recovered	Nymphs Released	Nymphs Inside	Nymphs Outside	Nymphs Engorged and Dropped	Total Nymphs Recovered
Cow 3	A (TX)	10	7	30	14	3	2	19
	B (OK)	10	4	30	6	0	6	12
	C (TX)	Control	0	15	1	5	0	6
	D (OK)	Control	0	15	0	0	0	0
	E (TX)	10	7	6	2	0	0	2
	F (OK)	10	5	6	0	0	0	0
	G (TX)	10	7	15	4	1	0	5
	H (OK)	10	8	15	0	1	0	1
					27	10	8	45

	Strain	Males Released	Males Recovered	Nymphs Released	Nymphs Inside	Nymphs Outside	Nymphs Engorged and Dropped	Total Nymphs Recovered
Totals	TX	90	75	198	76	22	23	121
	OK	90	54	198	18	8	21	47
	Total	180	129	396	94	30	44	168

Table 2: Recovery averages from preliminary investigation of aggregation attachment and attachment preferences of Gulf Coast tick, *Amblyomma maculatum* Koch, nymphs, Texas and Oklahoma strains on cattle.

Strain	% Males Recovered	% Nymphs Recovered Inside Ring	% Nymphs Recovered Outside Ring	% Nymphs Recovered Engorged and Dropped	Total Nymphs Recovered	% of Original Nymphs Recovered
TX	83	63	18	19	121	61
OK	60	38	17	45	47	24
Total	72	56	18	26	168	42

recovered outside the ring was greater than those recovered inside, if any were recovered at all. Except Cell E (Cow 1, TX strain), in every case when feeding adult males were present and presumably emitting AAAP, more nymphs were found inside the ring, if any were recovered at all. In only three cases (Cow 1, Cell H; Cow 3, Cell D; Cow 3, Cell F), no nymphs were recovered.

Discussion

The purpose of this preliminary experiment was to determine a satisfactory level of infestation of immature ticks in the cells need for the proposed aggregation study as well as to optimize the technique of using the stockinette material, inner cell, and counting method. Due to poor collection method and quality of data, an analysis of this study was not performed. However, several key details were garnered in regards to promoting our next study.

Except in one case, recovery of nymphs was always lower than the original infestation amount added to the cell. Often the number collected was much lower than the original released. Therefore, it was concluded that an infestation number of less than 30 nymphs might commonly lead to too few nymphs collected at the end of the experiment. The low recovery was probably due to two factors, 1) ticks are subject to r-selection and it is normal for most offspring to not survive to adult hood, even in the best environmental conditions and 2) nymphs may have escaped through the stockinette material that formed the cells prior to attachment. However, the total recovery of nymphs was encouraging, as 61% of Texas strain nymphs and 24% of Oklahoma strain were recovered (Table 2).

When the stockinette material was removed, the majority of hair that was glued to the material was also removed (Figure 9). This phenomenon created a third category of tick positioning when determining whether or not ticks had attached inside the inner cell area or outside area. The inner cell area is the area that contains the AAAP released by the male, making this new position an important consideration. This new position was labeled as “on the ring” in subsequent experiments. However, because the actual area of the new category “on the ring” was trivial compared to the overall area within the cell, I chose to group the “on the ring” with “in the ring”. The reasoning for grouping the “on the ring” category with “inside the ring” follows that the de facto area presumably treated with the pheromone (from feeding males) included the spaces underneath the glued stockinet where males would commonly wedge themselves. Also the grouping was chosen (“inside the ring” combined with “on the ring” instead of “on the ring” combined with “outside the ring”) because often adults were found along this edge before the inner ring was removed possibly releasing AAAP under the stockinette material, effectively making the “on the ring” area part of the AAAP area.

The method of counting the ticks in subsequent studies was determined in this preliminary study. Two methods were employed, visual and manual counts. After the stockinette material was removed, the cell was photographed, a print of the photograph was made and the ticks were then correspondingly marked on the print relative to their observed position; this being the visual method. Then, the ticks were manually counted and removed from the cell; this being the manual method. The numbers of nymphs collected were often slightly different from those visually recorded as present. This discrepancy was mainly due to the very small size (1-2mm) of the nymphs and their

common behavior of aggregating very closely while feeding; often overlapping in such a way as to obstruct an adjacent nymph visually (Figure 10), which wasn't a factor when removing ticks manually. However, the first observation with the photograph was deemed more accurate as to the location of each nymph prior to removal of the stockinette material. The differences between the two measurements were never more than 1; and it was noted that, once the cell material was removed, some nymphs would detach or move. The time between manual removal and the original opening of the cell was approximately 15 minutes, as compared to within 5 minutes with the observation. This time allowed for increased tick movement and was likely a larger source of error. Therefore, only the tick counts from marked photographs were used.

It was observed that the number of Texas strain nymphs recovered were much higher than the Oklahoma strain nymphs (Table 2). This may be due to the seasonality of the nymphs, but also may be due to overall fitness of individual nymphs. It has been often anecdotally noted in the Texas A&M Tick Research Laboratory that Oklahoma strain nymphs seem weaker than their Southern cousins. Unfortunately, this "weakness" was not accounted for in subsequent studies in regards to infestation concentrations of nymphs, but would have been advisable. Future studies should consider this observation when working with Oklahoma nymphs and ticks in general.

Nymphs were allowed to engorge for a period of 7 days during this study. When the cells were cut open and immature ticks were counted, many nymphs had already completely engorged and had dropped off (Table 1). Their attachment location could not be determined for these individuals and a large portion of data was lost. This source of error was accounted for in the subsequent studies where the attachment period was

Figure 10: Aggregation of Gulf Coast tick, *Amblyomma maculatum* Koch, nymphs in area where males are attached and emitting AAAP. Note how closely nymphs cluster together, hindering an accurate visual count.

limited to 5 days. Originally, the experimental design limited this period to 4 days, but after cell inspection, another day was added. Attachment periods should be between 4-5 days, and carefully monitored on the 4th day.

In creating a more efficient study the following points should be noted. In an attempt to recover enough nymphs for statistical analysis, at least 30 nymphs should be released per cell. If possible, a finer mesh stockinette material should be used in order to prevent the escape of nymphs before they attach, feed and engorge. The sparing use of adhesive to glue the inner cell to the hair will reduce the size of the third category of position "on the ring." The most efficient counting method is to remove stockinette material, photograph the cell immediately and visually mark positions onto the photograph from what is present in the cell. When using Oklahoma ticks, it is advisable to increase the number of immature ticks released into the cell. Allow the nymphs to feed for no more than 4 to 5 days in an attempt to avoid losing ticks that have engorged and dropped off the animal.

CHAPTER III

INTERACTION OF GULF COAST TICK, *Amblyomma maculatum* KOCH, NYMPHS, TEXAS STRAIN, ON CATTLE

The immature stages of the Gulf Coast tick and other tick species in general, are often the least understood stadia of the tick life cycle. On-host activities of developmental stages are poorly understood because the young are more difficult to observe, leading research to commonly target the adult stages, with surveillance and suppression techniques being no different. Hoogstraal and Aeschlimann (1982) defined adult and immature Gulf Coast ticks as having limited host groups (Table 3). Immature ticks are known to parasitize ground-dwelling birds and small rodents (Teel et al. 1988, Teel et al. 1998). As Uilenberg (1982) indicates, past publications have only anecdotally reported the preferred large mammalian hosts of immature ticks, such as cattle, sheep, goats, and horses (Hooker et al. 1912, Bishopp and Hixson 1936, Hixson 1940, Bishopp and Trembley 1945, Semtner and Hair 1973); however, these are all domestic species, are easily inspected and therefore, are likely to be the only source of information of host utilization in the literature. Adult ticks parasitize a wide range of large mammals, including cattle, white-tailed deer, and coyotes (Barker et al. 2004). No data exists on the host utilization of large mammals by immature ticks and few studies have attempted to address this issue (Barker et al. 2004). Furthermore, many Gulf Coast ticks, including immature ticks, reach peak activity during winter months when most hair coats are longest and identification is often difficult due to weather and coat length. Most significantly, there is no evidence that large host utilization by immature ticks does not occur throughout the range of the Gulf Coast ticks. It is the assumption of this study, as

well as anecdotally affirmed, that large hosts are utilized by immature ticks; it is the difficulty in identification that has prevented this from being proven thus far. The purpose of the current study was to demonstrate the on-host aggregation and attachment behavior of immature ticks of the Texas strain Gulf Coast tick in the presence of adult males, adult females and alone.

Materials and Methods

Although the procedure for this experiment is similar to the previous, there are a few nuances, which make describing the methodology worth repeating.

Texas and Oklahoma strain Gulf Coast ticks were used in this study. All ticks were fed on Leghorn chickens in the Tick Research Laboratory Veterinary Park of Texas A&M University, College Station, TX 77843, under approved Animal Use Protocol 2002-208, incubated at 23ºC, 85% RH, with a 14-hour photophase. Three cows were used in this experiment. The cows were housed in the Texas A&M Tick Research Laboratory. Cows were maintained in metabolism-like elevated stalls and provided with Calf Creep Pellet (Producers Cooperative Association, Bryan, TX 77803) twice daily and given free access to water (Figure 6). Cells were arranged on the back of each cow. Cells and labeling proceeded posteriorly with the first cell labeled “A” and the last labeled “F” (Figure 7). Temperature and humidity were maintained at 20-30ºC and 85-95% RH with a 12-hour photophase and monitored hourly with a Hobo® Pro Series (Model No. H08-032-08, Onset Computer Corporation, Pocasset, MA 02559-3450).

Where the cells were to be glued onto the hide, hair was clipped down to ¼-cm. The cell material used was 4 inch wide Tomac® tubular stockinette (American Hospital Supply, McGraw Park, Il 60085) glued to each host animal with livestock identification

tag cement (Nasco, Fort Atkinson, WI, 53538). Size 64 rubber bands (Alliance Rubber Company, Hot Springs, AR 71903) were used to close cells. The stockinette material was stretched to create a cell approximately 6-in. in diameter (15.24 cm).

A small cell, roughly 2 in. (5.08 cm) in diameter, of 2. in wide Tomac® tubular stockinette (American Hospital Supply, McGraw Park, Il 60085) was glued to each host animal with livestock identification tag cement in the center of each the aforementioned 6-in cell (Figure 7). The smaller cells were closed in with rubber bands.

One treatment (males) and two controls (females and empty), were compared. Females were used as a second control to evaluate the response of nymphs to the presence of adults without the presence of the AAAP pheromone. It is commonly known that only males emit AAAP among Gulf Coast ticks, but females do release other pheromones. Secondarily, the use of females would indicate whether or not the nymphs were responding to feeding alone or even an area of increased inflammation on the host animal. Ten adults were used per cell in the male treatment and female control. Each group of adults was placed in each 2-in. cell, and forced to attach and feed (Figure 7). The smaller cells were used to isolate male and female ticks within the center of each cell. The inner cell was removed simultaneously with the infestation of nymphs. The inner cell was removed by gently pulling from the hair, which created a ring of bare, exposed skin (Figure 9). Until this time, the adults were forced to attach and feed in the center of the cell. The predetermined period of adult attachment of 5 days was selected based on preliminary results indicating that adult males reach optimal pheromone output after 4-8 days. Thus, when the inner cell was removed and nymphs were released within the cell, the pheromone attractant emanated from the center of the cell. Each control cell

also contained the smaller cells in an attempt to mimic any conditions such cells would create during the removal process of the smaller cells.

Nymphs were released into the cell on the 6th day after the infestation of the adults. Nymphs were released into the cell via an eppendorf tube. The eppendorf tube was gently shaken to cluster the nymphs and then inverted into the center of the cell.

The experiment was completed on day 10, the 5th day after the release of the nymphs. On the morning of the 5th day after nymphs had been released into each cell, the stockinette material of the outer cell was cut with scissors to the level of the hair and observations were made. Nymphs and adults were counted as attached "inside the ring," "on the ring of skin," or "outside the ring."

Nymphs and adults were counted by two methods, visually and manually. A Nikon Coolpix5000 (Nikon Corporation, Tokyo, JAPAN) camera was used to photograph the cell (on the last day only) and the locations of each nymph or adult were then marked onto a printout of that photograph. After the visual count and record with the camera was completed, the cell was then manually cleared and counted of both immature and adult ticks.

Three simultaneous infestations occurred, utilizing three cows (Figure 11). This provided a block design where cells were treatment replicates, each cow was a block, and all three cows completed a random block design. The replication source for this experiment was the cow, with three replicates total. Infestation among cells was not randomized because the influence between cells was a concern in this experiment. Adult

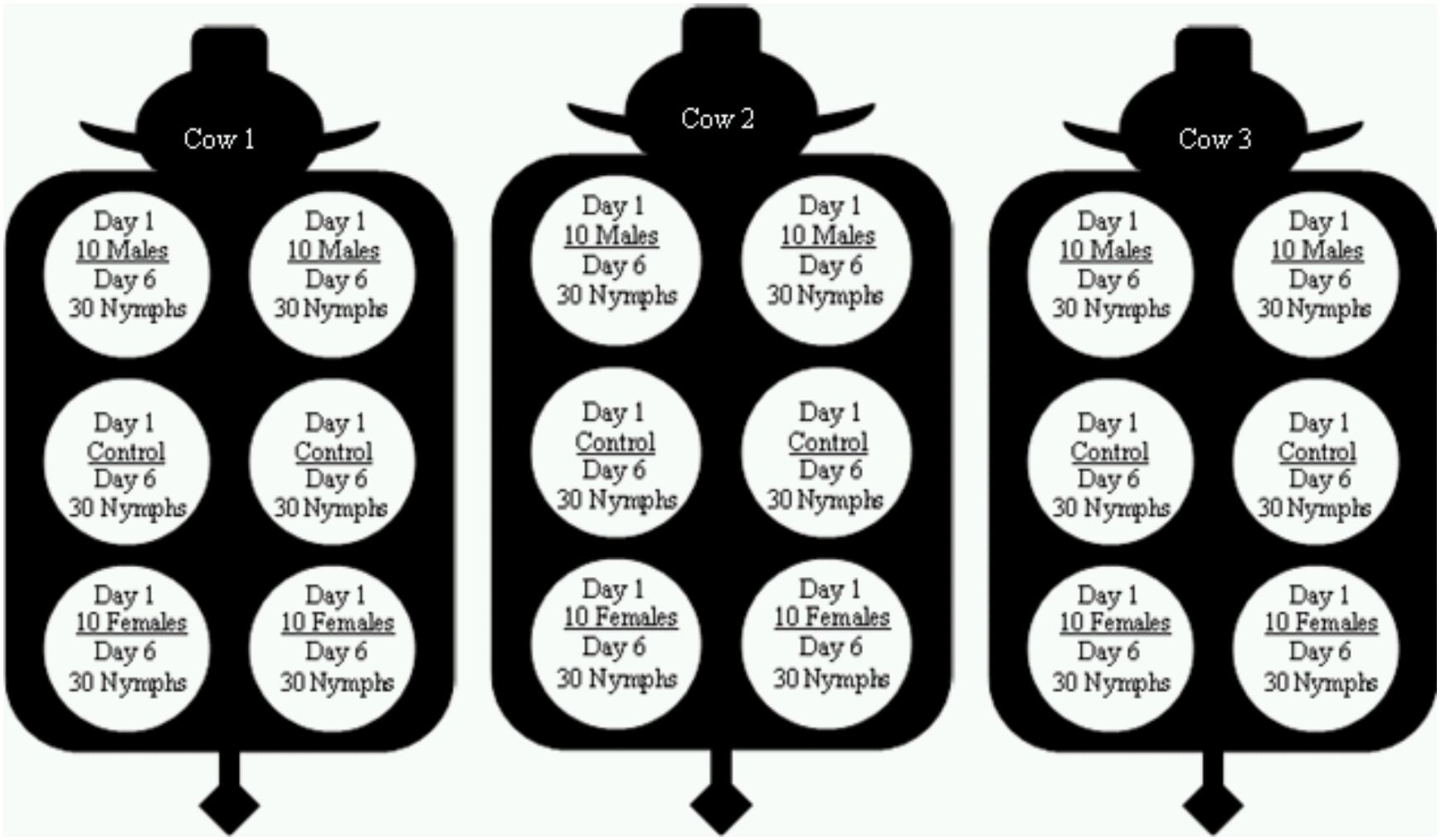

Figure 11: Experimental design of interaction of Gulf Coast ticks, *Amblyomma maculatum* Koch, nymphs, Texas strain on cattle.

males are known to influence the attachment behavior of females and males, and therefore must be kept separate.

Statistical Methods

Statistical analyses were performed using SAS® System for Windows v 9.0. Two different analyses were performed, the mortality among nymphs in the presence of different treatment groups and the preferred attachment position of nymphs in the presence of different treatments (i.e. the presence or absence of AAAP). The Cochran-Mantel-Haenszel Statistic (controlling for cow) was used to demonstrate significant differences in general association among treatment groups, as well as to produce a 95% confidence interval using the odds ratio (Stokes et al. 1995). Data was categorized creating a table that examined the homogeneity of proportions where treatment type was not random, but the response was. The variable cow is a blocking variable and although it was not of significance to the study, it was considered as a possible effect on the analysis. Because the statistical significance among cows was insignificant, the data was pooled and an overall statistic was produced.

Results

Mortality Status

In determining mortality, the independent variable is the "type of treatment" (control, male, female), and the response variable is "mortality status" ("survivors" i.e. alive/feeding and "presumed dead" i.e. dead or unaccounted). There are significant differences in the proportions of survivors in the three types of treatments, p-value of less than 0.0001 (Table 3). Sub-dividing the treatments into pairs of analyses revealed that

Table 3: Texas strain Gulf Coast tick, *Amblyomma maculatum* Koch, summary statistics for treatment by survivors, controlling for cow.

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	54.4950	<.0001
2	Row Mean Scores Differ	2	61.5203	<.0001
3	General Association	2	61.5203	<.0001

Total Sample Size = 273

the statistical difference in survivor proportions between control and female was negligible, with a p-value of 0.1751. However, the statistical difference between control and male (p-value < 0.0001) and female and male (p-value < 0.0001) were both statistically significant (Table 4). These data demonstrated that presence of attached adult males emitting AAAP decrease immature tick mortality with a general association significance p-value of less than 0.0001.

Concerning recovery rates, of 273 nymphs released into nine separate treatment cells on three cows, 58% were recovered (Table 5). The treatment cells with the attached adult male Gulf Coast ticks had the lowest mortality rate among nymphs with an overall recovery rate of 53%, while the highest mortality of the treatments was among the control cells (i.e. no adults attached), with an overall recovery rate of 21%. The female adult treatment cells (a secondary control) had a slightly higher recovery rate than the control cells, i.e. just over 26% overall. Although the recovery rates differed from cow to cow, actual recovery of nymphs among the male treatments on all cows ranged from 70% to 100%, while actual recovery of nymph among the control treatments on all cows ranged from 27% to 53%. Actual recovery of nymphs from female treatments on all cows ranged from 40% to 57%.

Position

In determining tick nymph position (either inside or outside the ring), treatment is the independent variable and attachment position is the response variable. The analysis on the effect of type of treatment on attachment position demonstrated that there were statistical differences among all treatment groups (p-value < 0.0001) (Table 6). Further analysis revealed that the statistical difference between

Table 4: Texas strain Gulf Coast tick nymphs, *Amblyomma maculatum* Koch, summary statistics for treatment by survivors, controlling for cow 1) control vs. male, 2) control vs. female, 3) female vs. male.

1)

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	59.1293	<.0001
2	Row Mean Scores Differ	1	59.1293	<.0001
3	General Association	1	59.1293	<.0001

Estimates of the Common Relative Risk (Row1/Row2)

Type of Study	Method	Value	95% Confidence Limits	
Case-Control (Odds Ratio)	Mantel-Haenszel	19.1658	7.6583	47.9647
	Logit **	10.4349	3.9491	27.5729

Total Sample Size = 183

2)

Cochran-Mantel-Haenszel Statistics (Modified Ridit Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	1.8388	0.1751
2	Row Mean Scores Differ	1	1.8388	0.1751
3	General Association	1	1.8388	0.1751

Estimates of the Common Relative Risk (Row1/Row2)

Type of Study	Method	Value	95% Confidence Limits	
Case-Control (Odds Ratio)	Mantel-Haenszel	1.4991	0.8305	2.7060
	Logit	1.5077	0.8193	2.7746

Total Sample Size = 180

3)

Cochran-Mantel-Haenszel Statistics (Modified Ridit Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	40.0693	<.0001
2	Row Mean Scores Differ	1	40.0613	<.0001
3	General Association	1	40.0532	<.0001

Estimates of the Common Relative Risk (Row1/Row2)

Type of Study	Method	Value	95% Confidence Limits	
Case-Control (Odds Ratio)	Mantel-Haenszel	8.8058	3.9886	19.4406
	Logit **	4.1138	1.6022	10.5628

Total Sample Size = 183

Table 5: Recovery data from of study of interaction of Gulf Coast ticks, *Amblyomma maculatum* Koch, nymphs, Texas strain on cattle.

Cow	Treatment	Response Level - Survivors		Total	Cell Name	% Survived
		Survivors (Recovered)	Presumed Dead			
lex	control	16	14	30	A	53
lex	male	30	0	30	B	100
lex	female	12	18	30	D	40
rex	control	9	21	30	A	30
rex	male	21	9	30	B	70
rex	female	17	13	30	D	57
ted	control	8	22	30	A	27
ted	male	33	0	33	B	100
ted	female	13	17	30	D	43
		159	114	273		58

Table 6: Texas strain Gulf Coast tick nymphs, *Amblyomma maculatum* Koch, summary statistics for treatment by position, controlling for cow, using general association.

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	36.8914	<.0001
2	Row Mean Scores Differ	2	37.6392	<.0001
3	General Association	2	37.6392	<.0001

Total Sample Size = 159

control and female was negligible, with a p-value of 0.0856 (Table 7). However, the statistical differences between control and male (p-value < 0.0001) and female and male (p-value < 0.0001) were both statistically significant. These data demonstrated that presence of attached adult males emitting AAAP elicits an attraction-aggregation-attachment response from immature ticks, with a general association significance p-value of less than 0.0001.

The comparison of preferred tick aggregation position within the cells among the treatments demonstrated that nymphs were approximately six times more likely to be found inside the ring of bare skin than outside, within the cell, (Table 8, Figure 12) if adult males were present. This is contrasted with control cells (no adults) where nymphs were approximately two times less likely to be found in the condensed treatment area. Nymphs in the female treatment cells were insignificantly more likely to be found outside than inside the condensed treatment area.

Discussion

Data indicate that mortality status among immature Texas strain Gulf Coast ticks differs across the treatment types. The null hypothesis that mortality status is the same for all of treatment type is thus rejected; the high degree of significance of general association (p-value less than 0.0001) among all three treatments (male, control, female) demonstrates this rejection. Upon further examination, there is no significant difference between the control and the female treatment on mortality, but the difference between the control and male treatment is significant as is the difference between the female and male treatment. Because it is known that females do not emit AAAP, this study suggests a

Table 7: Texas strain Gulf Coast tick nymphs, *Amblyomma maculatum* Koch, summary statistics for treatment by position, controlling for cow 1) control vs. male, 2) control vs. female, 3) female vs. male.

1)

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	34.1348	<.0001
2	Row Mean Scores Differ	1	34.1348	<.0001
3	General Association	1	34.1348	<.0001

Estimates of the Common Relative Risk (Row1/Row2)

Type of Study	Method	Value	95% Confidence Limits	
Case-Control (Odds Ratio)	Mantel-Haenszel	0.0986	0.0393	0.2476
	Logit **	0.1138	0.0392	0.3300

Total Sample Size = 117

2)

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	2.9555	0.0856
2	Row Mean Scores Differ	1	2.9555	0.0856
3	General Association	1	2.9555	0.0856

Estimates of the Common Relative Risk (Row1/Row2)

Type of Study	Method	Value	95% Confidence Limits	
Case-Control (Odds Ratio)	Mantel-Haenszel	0.4068	0.1466	1.1286
	Logit **	0.4789	0.1679	1.3659

Total Sample Size = 75

3)

Cochran-Mantel-Haenszel Statistics (Based on Table Scores)

Statistic	Alternative Hypothesis	DF	Value	Prob
1	Nonzero Correlation	1	17.4134	<.0001
2	Row Mean Scores Differ	1	17.4134	<.0001
3	General Association	1	17.4134	<.0001

Estimates of the Common Relative Risk (Row1/Row2)

Type of Study	Method	Value	95% Confidence Limits	
Case-Control (Odds Ratio)	Mantel-Haenszel	0.1895	0.0794	0.4522
	Logit	0.2066	0.0797	0.5352

Total Sample Size = 126

Table 8: Position data from study of interaction of Gulf Coast ticks, *Amblyomma maculatum* Koch, nymphs, Texas strain on cattle.

Cow	Treatment	Response Level - Position		Total
		Inside	Outside	
lex	control	6	10	16
lex	male	27	3	30
lex	female	7	5	12
rex	control	5	4	9
rex	male	15	6	21
rex	female	10	7	17
ted	control	0	8	8
ted	male	32	1	33
ted	female	5	8	13
		83	76	159

Cell Name	% In / Out
A	38 / 62
B	90 / 10
D	58 / 42
A	56 / 44
B	71 / 29
D	59 / 41
A	0 / 100
B	97 / 3
D	38 / 62
	58

Figure 12: Comparison of position of Gulf Coast ticks, *Amblyomma maculatum* Koch, nymphs in cells by treatment. From left to right, 15 nymphs inside vs. 6 outside male treatment present. Five nymphs inside vs. 8 outside female treatment. Zero nymphs inside vs. 8 nymphs outside control cell.

theory that AAAP (from males) encourages survival among immature tick stages by fostering attachment and feeding with some method that increases tick survival rates.

It is also evident from the data that there is a correlation between treatment type and localization of nymphs to specific positions on the host. The null hypothesis that the presence of males does not affect the positional location of nymphal ticks is thus also rejected. Again, the analysis is substantiated by the parallel data of control and female treatments; while there is a high degree of significance between males and controls and males and females, there is little between controls and females. Because it is known that females do not emit AAAP, this study supports the theory that AAAP (emitted by males) elicits an attraction-aggregation-attachment response. Therefore, the conclusion that among Texas strain Gulf Coast ticks, AAAP emitted by adult males does influence immature tick behavior, eliciting an attraction-aggregation-attachment response.

The implications of these data support the concept of immature Gulf Coast tick surveillance tactics using an AAAP extract or synthetic pheromone. These data are crucial in developing such techniques of tick surveillance and management.

Nymphal tick recovery rates of this study (58%) were high in comparison to other studies, which were typically under 20% (Riek 1962, Williams 2002). This study did not employ a free release method as in previous studies, but used an artificially-forced environment already on the cow. This artificial condition yields higher recovery rates. In order to produce more meaningful results, further studies require not only an artificial technique with high recovery rates, but also a free release technique that yields a substantially higher recovery rates than the norm of less than 20% (Williams 2002).

As multitudes of immature ticks are not known to attack hosts in the field and feed on large ruminants only anecdotally, this study chose to keep cell infestation numbers no higher than thirty. Each cell was infested with thirty nymphs. A smaller number of nymphs could have provided a standard mean and error for each cell. However, in preliminary tests, often the number of nymphs collected at the termination of the experiment was much less than the number released. A reasonable amount of time to observe and measure ticks was also a consideration in choosing infestation numbers within cells, where increasing numbers of nymphs considerably hampers locating and recording their positions.

The method of recording the tick positions was done by visual and manual methods. These two measurements were taken in an attempt evaluate the accuracy of each method. During preliminary trials, numbers of nymphs collected at the end of the period often differed slightly from those visually recorded. This discrepancy was mainly due to the very small size (1-2 mm) of the nymphs and their common behavior of aggregating very closely while feeding; often overlapping in such a way as to obstruct an adjacent nymph visually, but not manually. However, the first observation (the photograph) was deemed more accurate as to the location of each nymph prior to disturbance by removing stockinette material; it was also noted, that once the cell material was removed, that some nymphs would detach or move. Therefore, only the tick counts from the marked photograph were used.

The ring of bare skin created after the removal of the inner cell used to isolate adult males and females created a third category of “on the ring” when counting nymphs and their position (Figure 9). Although the creation of the ring established a useful

marker for whether or not a nymph had attached in an area with APP, it did create a problem for the analysis. Therefore, a judgment call was required as to whether or not the “on the ring” category should be included in the “outside the ring” position or “inside the ring” position. This study advocates that the “on the ring” category should be included with the “inside the ring” category for a number of reasons including its small size in comparison to the rest of the cell, specifically the “outside the ring” area. Reasonably speaking, the likelihood that a nymph would randomly attach on the ring was small in comparison to outside the ring. Furthermore, the adult ticks commonly chose this area to attach, feed and release AAAP. Therefore it was considered that this area was “contaminated” with AAAP, and therefore different from the outer area and similar to the inner area. As a precaution, the same analysis of attachment was done with the “on the ring” category included with the “outside the ring.” This only slightly reduced the significance, but yielded a similar outcome. However, in future studies a more precise method, possibly eliminating the “on the ring” category, would be prudent.

CHAPTER IV

CONCLUSIONS

Few studies have addressed surveillance techniques incorporating the biology of immature ticks. The relative ease of working with adult ticks, compared to immature ticks, in the laboratory or field, has narrowed research efforts. Immature tick behavior may reveal a new focus in the ability to control tick populations in general. It has been demonstrated by this study that immature ticks are influenced in a similar fashion as adult ticks, by adult ticks which have released AAAP. Immature ticks, nymphs specifically of Texas strain Gulf Coast ticks have been shown to not only aggregate and attach to a host while in the presence of feeding adult males releasing AAAP, but also to reduce their overall mortality.

Although this study has not contributed to the knowledge of whether or not immature ticks actually attack large mammalian host, it has provided a conceptual tool in the development of techniques to monitor the alleged, but historically difficult to prove, behavior that immature ticks attack and feed on large hosts.

The ever increasing threat of heartwater, as well as traditionally-occurring hindrances of Gulf Coast ticks, urges the need for new tools in surveillance and control of Gulf Coast ticks. This study provides a basis for that research.

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APPENDIX I

SAS system statements using Mantel-Haenszel correlation statistic PROC FREQ used for this study. Data set nomenclature "GCTTX_survivors_vs_dead_" refers to strain of Gulf Coast tick ("GCTTX_") and analysis performed ("survivors_vs_dead_"). The last section of each statement refers to the treatments being compared.

```
data GCTTX_survivors_vs_dead_RxALL;
    length treatment $11. ;
    input cow $ treatment $ survivors $ count @@;
    cards;
lex control recovered 16    lex control lost    14
lex male    recovered 30    lex male    lost    0
lex female  recovered 12    lex female  lost    18
rex control recovered 9     rex control lost    21
rex male    recovered 21    rex male    lost    9
rex female  recovered 17    rex female  lost    13
ted control recovered 8     ted control lost    22
ted male    recovered 33    ted male    lost    0
ted female  recovered 13    ted female  lost    17
;
proc freq ;
    weight count;
    tables cow*treatment*survivors /cmh chisq measures;
    tables cow*treatment*survivors /cmh scores=modridit;
run;
```

```
data GCTTX_survivors_vs_dead_RxFC;
    length treatment $11. ;
    input cow $ treatment $ survivors $ count @@;
    cards;
lex control recovered 16    lex control lost    14
lex female  recovered 12    lex female  lost    18
rex control recovered 9     rex control lost    21
rex female  recovered 17    rex female  lost    13
ted control recovered 8     ted control lost    22
ted female  recovered 13    ted female  lost    17
;
proc freq ;
    weight count;
    tables cow*treatment*survivors /cmh chisq measures;
    tables cow*treatment*survivors /cmh scores=modridit;
run;
```

```
data GCTTX_in_on_vs_out_RxFM;
    length treatment $11. ;
    input cow $ treatment $ position $ count @@;
    cards;
lex male    inside  27  lex male    outside 3
lex female  inside  7   lex female  outside 5
rex male    inside  15  rex male    outside 6
rex female  inside  10  rex female  outside 7
ted male    inside  32  ted male    outside 1
ted female  inside  5   ted female  outside 8
;
proc freq ;
    weight count;
    tables cow*treatment*position /cmh chisq measures;
    tables cow*treatment*position /cmh scores=modridit;
run;
```

```
data GCTTX_survivors_vs_dead_RxCM;
    length treatment $11. ;
    input cow $ treatment $ survivors $ count @@;
    cards;
lex control recovered 16    lex control lost    14
lex male    recovered 30    lex male    lost    0
rex control recovered 9     rex control lost    21
rex male    recovered 21    rex male    lost    9
ted control recovered 8     ted control lost    22
ted male    recovered 33    ted male    lost    0
;
proc freq ;
    weight count;
    tables cow*treatment*survivors /cmh chisq measures;
    tables cow*treatment*survivors /cmh scores=modridit;
run;
```

APPENDIX II

SAS system statements using Mantel-Haenszel correlation statistic PROC FREQ used for this study. Data set nomenclature "GCTTX_survivors_vs_dead_" refers to strain of Gulf Coast tick ("GCTTX_") and analysis performed ("survivors_vs_dead_"). The last section of each statement refers to the treatments being compared.

```
data GCTTX_in_on_vs_out_RxALL;
    length treatment $11. ;
    input cow $ treatment $ position $ count @@;
    cards;
lex control inside  6   lex control outside 10
lex male    inside  27  lex male    outside 3
lex female  inside  7   lex female  outside 5
rex control inside  5   rex control outside 4
rex male    inside  15  rex male    outside 6
rex female  inside  10  rex female  outside 7
ted control inside  0   ted control outside 8
ted male    inside  32  ted male    outside 1
ted female  inside  5   ted female  outside 8
;
proc freq ;
    weight count;
    tables cow*treatment*position /cmh chisq measures;
    tables cow*treatment*position /cmh scores=modridit;
run;
```

```
data GCTTX_in_on_vs_out_RxCF;
    length treatment $11. ;
    input cow $ treatment $ position $ count @@;
    cards;
lex control inside  6   lex control outside 10
lex female  inside  7   lex female  outside 5
rex control inside  5   rex control outside 4
rex female  inside  10  rex female  outside 7
ted control inside  0   ted control outside 8
ted female  inside  5   ted female  outside 8
;
proc freq ;
    weight count;
    tables cow*treatment*position /cmh chisq measures;
    tables cow*treatment*position /cmh scores=modridit;
run;
```

```
data GCTTX_survivors_vs_dead_RxFM;
    length treatment $11. ;
    input cow $ treatment $ survivors $ count @@;
    cards;
lex male    recovered 30    lex male    lost    0
lex female  recovered 12    lex female  lost    18
rex male    recovered 21    rex male    lost    9
rex female  recovered 17    rex female  lost    13
ted male    recovered 33    ted male    lost    0
ted female  recovered 13    ted female  lost    17
;
proc freq ;
    weight count;
    tables cow*treatment*survivors /cmh chisq measures;
    tables cow*treatment*survivors /cmh scores=modridit;
run;
```

```
data GCTTX_in_on_vs_out_RxCM;
    length treatment $11. ;
    input cow $ treatment $ position $ count @@;
    cards;
lex control inside  6   lex control outside 10
lex male    inside  27  lex male    outside 3
rex control inside  5   rex control outside 4
rex male    inside  15  rex male    outside 6
ted control inside  0   ted control outside 8
ted male    inside  32  ted male    outside 1
;
proc freq ;
    weight count;
    tables cow*treatment*position /cmh chisq measures;
    tables cow*treatment*position /cmh scores=modridit;
run;
```

VITA

Aaron Wexler was born in Leuven, Belgium, to a family that stressed the value of education, family and dedication to one's life goals and happiness above all else. As a child, his interests included creativity, appreciation of the natural world, and how to meld into its mysteries. To Aaron's great fortune, his family was not only composed of an international mix, but also enjoyed *visiting* geographically distant relatives and traveling the world, always in an educational context.

Aaron attended the George Washington University, in Washington DC, graduating in 2000 with a B.S. in Biology. His achievements during his time at GWU included teaching the comparative vertebrate anatomy laboratory of his mentor, Dr. David Atkins, publishing an independently profitable city newsletter focusing on dog ownership in Washington, DC and developing and implementing a business plan to start a tour company where he prepared and led customized tours of Africa. Paramountly, he met his wife, Ngozi Uzogara.

Texas A&M presented many challenges and opportunities, particularly in continuing education. Aaron enjoyed the opportunity to work closely under the supervision of dedicated educators as a teaching assistant for Introductory Entomology and Insect Physiology, participated in outreach for local grammar school children, and received a certification in teaching excellence from Graduate Teaching Academy.

During his time at A&M, Aaron was able to lead 3 extended journeys to Africa, 1 month in Morocco, 3 months in East Africa and 2 months in Madagascar. He plans on continuing his interests in education and Africa. Aaron is reachable at 412 Minnie Bell Heep Building, Dept. of Entomology, College Station, TX 77843-2475.